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Influence of Antioxidants and the CYP1A1 Isoleucine to Valine Polymorphism on the Smoking - Lung Cancer Association

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Abstract. *To evaluate the association between CYP1A1 genotype and lung cancer risk and to assess the effect of CYP1A1 genotype and antioxidant supplementation on the smoking - lung cancer relationship we conducted a case-control study nested within a large cancer prevention trial cohort. Controls (n=324) were matched to cases (n=282) on age (± 5 years), intervention group and study clinic in a 1:1 ratio, using incidence density sampling. Genotype was determined by a PCR-based method and logistic regression was used to calculate relative risk estimates. Overall, we found no association between CYP1A1 genotype and lung cancer risk. CYP1A1 genotype did not modify the effect of smoking on lung cancer risk. However, in an examination of subgroups defined by randomized intervention assignment our findings suggest that alpha-tocopherol supplementation may reduce the risk of lung cancer associated with cumulative smoking exposure regardless of CYP1A1 genotype with the greatest effect seen among those with the variant CYP1A1 allele.*

Although environmental and industrial exposures such as radon, can increase lung cancer risk, it is well established that tobacco smoke is principally responsible for the incidence of this malignancy. However, not all persons exposed to high concentrations of airborne carcinogens develop lung cancer. Differences in lung cancer risk among individuals with similar carcinogent exposures may be due to genetic make-up and diet, which have been shown to modulate or neutralize carcinogen derived oxidative radicals.

The risk of developing cancer is determined by both the number and nature of cumulative carcinogenic exposures and by individual genetic variations (1). For example, genetic polymorphisms in detoxification enzymes can substantially alter the metabolic activation and the ultimate elimination of carcinogenic substances. Most pro-carcinogens are first metabolically activated by phase I enzymes (the cytochrome P450s such as CYP1A1) to exert their tumorigenic potential. The activated carcinogens are then detoxified by the phase II enzymes, such as glutathione S-transferases and N-acetyl transferases.

Aryl hydrocarbon hydroxylase, or CYP1A1, is believed to be responsible for the carcinogenic activation of benzo-(a)pyrene and other polyaromatic hydrocarbons in cigarette smoke. Cigarette smoking has been correlated with increased CYP1A1 expression. An isoleucine to valine substitution polymorphism in the heme-binding region of CYP1A1 has been associated with increased enzyme activity and thus may contribute to increased cancer risk (2). In this study, we explored the association between the CYP1A1 isoleucine to valine polymorphism and lung cancer risk in a nested case control study within a cohort of Finnish smokers who participated in a large cancer prevention trial. We also investigated whether CYP1A1 genotype could modify the effect of smoking behavior and the trial intervention on lung cancer risk.

Patients and Methods

The cases and controls for this study were selected from the cohort of the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study, a randomized, placebo-controlled prevention trial designed to determine whether alpha-tocopherol (50mg/day), beta-carotene (20

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Abbreviations: ATBC Study, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; CYP1A1, Cytochrome P450 1A1; GST, Glutathione s-transferase.

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Table I. *Medians and percent distributions of selected subject chara-cteristics by case - control status*[1].

Characteristic	Cases (n = 282)	Controls (n = 324)	P-value[2]	Odds ratio[3]
Age (years)	60.0	59.0	0.934	
BMI (Kg/m^2)	25.3	26.0	<0.04	
Alcohol (grams/day)	8.9	9.6	0.854	
Years smoked	40.0	39.0	<0.001	
Age started smoking	18	19	<0.001	
Serum alpha-tocopherol (mg/l)	11.4	11.9	0.05	
Serum beta-carotene (μg/l)	198.3	205.7	0.12	
CYP1A1 genotype				
WT/WT	243 (86%)	273 (84%)		1.00 (Ref)
WT/VT	36 (12.8%)	48 (14.8%)	0.759	0.84 (0.53-1.34)
VT/VT	3(1.1%)	3 (0.9%)		1.12 (0.23-5.62)

[1] Based on unmatched data with continuous variables expressed as the median.
[2] P-values as determined by Wilcoxon rank-sum tests and Chi^2 for categorical variables.
[3] Risk estimates from univariate conditional logistic regression.

mg/day), or both would reduce the incidence of lung, prostate and other cancers. The overall design, rational, and objectives of this study have been published, as have the main trial findings (3,4). The trial was conducted between 1985 and 1993 in southwestern Finland as a joint project between the National Public Health Institute of Finland and the National Cancer Institute of the United States. Potential participants, all men, aged 50-69 years residing in southwestern Finland (n=290,406) were identified from the computer list of the national population registry of Finland. To be eligible, the participants (29,133) had to be a current smoker of five or more cigaretts per day and willing to participate and give informed consent.

Selection of cases and controls. The cases consisted of 362 men, diagnosed with primary lung cancer (1CD-9: 162) during the years 1985-94 among those who gave a whole blood sample. Using incidence density sampling, the controls were selected from cohort participants who were alive and free of cancer at the time the matched case was diagnosed. Controls were matched to cases, by age (± 5 years), intervention group and study clinic in a 1:1 ratio.

Genotype analyses. DNA was isolated as previously described [5] and CYP1A1 genotype determined by a polymerase chain reaction based approach. Briefly, PCR fragments were amplified from DNA using specific sense and antisense primers. The variant alleles were detected by digestion with the restriction enzyme NCO1 and visualized by agarose gel electrophoresis [3].

Genotyping results were reviewed independently by two investigative groups. Assays were performed in batches containing restriction enzyme cutting controls and negative controls (PCR reagents without DNA). All laboratory personnel were blind to the case-control status of the samples. A random sample of 10% was repeated for quality control and revealed a 100% concordance rate. Genotyping was successful for 282 cases and 324 controls.

Statistical analyses. The Chi-square test for heterogeneity was used to test the hypothesis that the distribution of allele prevalences was the same for cases and controls. Conditional logistic regression techniques were used to examine the association between genotype and lung cancer risk. The heterozygous CYP1A1 individuals were pooled with the homozygous variant individuals due to the low prevalence of the latter and reports that even one variant allele results in increased lung cancer risk (6). Modification of the effect of genotype by age, tobacco and alcohol consumption on lung cancer risk was examined by statistical tests of the first order interaction term in the logistic regression models. We examined the association between cigarette smoking and lung cancer stratified by genotype in an unmatched analysis (using unconditional logistic regression techniques) to avoid the loss of subjects due to splitting of matched sets that fell into different strata of genotype. All unconditional logistic regression models were adjusted for the original matching criteria (age, intervention and study clinic). Tertiles of exposure for cigarette smoking were created using the distribution of smoking variables among the controls. Indicator variables were used to define the second and third tertiles of cigarette smoke exposure with the lowest tertile as the reference group. Continuous variables of cigarette smoke exposure were used to conduct linear trend tests.

Potential confounding of the association between the cigarette smoke exposure and cancer risk by other related risk factors was explored using Spearmar rank correlation analysis and multivariate logistic regression models, including stepwise regression models both before and after stratification by genotype and intervention assignment. If the potential confounder caused a significant change in the log likelihood estimate ($p<0.05$) and a greater than 10% change in the beta-coefficient, it was kept in the model for further multivariate analysis. Exclusion of cases diagnosed before blood draw did not materially alter any of the risk estimates. All analyses were performed using the statistical software package STATA (STATA Corporation, TX, USA).

Results

Table I shows a case-control comparison of selected subject characteristics. As expected, cigarette smoking and age started smoking were significantly different in the cases compared to controls. Serum alpha-tocopherol and beta-carotene levels were slightly lower in the cases than controls

Table II. *Risk of lung cancer by tertiles of years smoked stratified by CYP1A1 genotype and intervention assignment*[1].

		Years Smoked (Tertiles)			
		<37	37-42	>42	
Group	Genotype	OR (95% CI) #case/ #controls	OR (95% CI) #case/ #controls	OR (95% CI) #case/ #controls	P-trend[2]
All subjects	WT/WT	1.0 (ref) 54/93	1.83 (1.12-3.01) 83/92	3.11 (1.67-5.81) 106/88	<0.001
	WT/VT + VT/VT	1.0 (ref) 10/23	2.52 (0.79-8.08) 14/15	3.69 (0.71-19.10) 15/13	0.055
Alpha- Tocopherol					
No	WT/WT	1.0 (ref) 22/58	3.22 (1.58-6.59) 44/47	7.09 (2.93-17.15) 59/38	<0.001
	WT/VT + VT/VT	1.0 (ref) 4/14	21.71 (2.16-218.53) 8/4	21.95 (1.35-357.96) 7/8	0.01
Yes	WT/WT	1.0 (ref) 32/35	0.97 (0.47-1.98) 39/45	1.15 (0.45-2.96) 47/50	0.12
	WT/VT + VT/VT	1.0 (ref) 6/9	0.82 (0.18-3.75) 6/11	2.16 (0.27-20.02) 8/5	0.97
Beta-carotene					
No	WT/WT	1.0 (ref) 27/46	1.72 (0.84-3.51) 38/43	2.75 (1.07-7.02) 44/37	0.01
	WT/VT + VT/VT	1.0 (ref) 4/14	3.92 (0.67-22.83) 7/7	3.72 (0.43-32.37) 4/5	0.5
Yes	WT/WT	1.0 (ref) 27/47	2.14(1.06-4.29) 45/49	4.19(1.76-9.98) 62/51	<0.001
	WT/VT + VT/VT	1.0 (ref) 6/9	1.80 (0.33-9.74) 7/8	4.82 (0.31-74.32) 11/8	0.006

[1]All unmatched logistic regression models adjusted for age at randomization, study clinic and daily cigarettes smoked.
[2]p for trend based upon the p-value of smoke years modeled as a continuous term.

with tocopherol reaching a level of marginal statistical significance. The distribution of the CYP1A1 alleles was not different comparing the cases to controls and there was also no association between CYP1A1 genotype and lung cancer risk. In addition, CYP1A1 genotype was not associated with any of the histological subtypes of lung cancer (data not shown).

Table II shows the association between years of cigarette smoking and lung cancer risk stratified by CYP1A1 genotype. CYP1A1 genotype had no apparent overall effect on the association between years smoked and lung cancer risk, with relative risks between three and four for the long term smokers compared to those with fewer years of smoking. The trend of increasing lung cancer risk with years smoked was statistically significant for both the CYP1A1 genotype categories.

Table II also shows the association between years smoked and lung cancer risk stratified by intervention assignment and the two CYP1A1 genotype categories. This stratified analysis was performed although the interaction terms were not significant because alpha-tocopherol may reduce the risk of lung cancer (7), while beta-carotene has been shown to increase the risk of lung cancer (3). The intervention assignment categories shown as beta-carotene in table II include, both the beta-carotene intervention group and the combined alpha-tocopherol and beta-carotene supplementation groups. The no beta-carotene group includes both the placebo and the alpha-tocopherol groups. The intervention groups were collapsed because there were no differences in the risk estimates between the beta-carotene and the alpha-tocopherol and beta-carotene groups or the placebo and alpha-tocopherol groups (data not shown). Conversely, the intervention assignment category shown as alpha-tocopherol includes both the tocopherol intervention group and the alpha-tocopherol and beta-carotene supplementation groups. The no tocopherol group includes both the placebo and beta-carotene groups. The intervention groups were collapsed in this case because there were no

differences in the risk estimates between tocopherol and the alpha-tocopherol and beta-carotene groups or the placebo and beta-carotene groups (data not shown). The combining of intervention assignment groups also helped improve numbers in each stratum.

Among the study participants supplemented with beta-carotene there was an increase in lung cancer risk in both the genotype categories on comparing those in the highest tertile of years smoked to the lowest tertile. Genotype apparently did not alter the lung cancer - years smoked relationship. However, the risk of lung cancer due to years smoked was somewhat greater among the beta-carotene supplemented individuals than the non-supplemented individuals.

Among the study participants that were not supplemented with tocopherol, the relative risk of lung cancer was seven in the CYP1A1 wt/wt group and twenty-two in the wt/vt + vt/vt group comparing those in the highest tertile of years smoked to the lowest tertile. Conversely, in the tocopherol supplemented group the relative risks were reduced to one and two in the CYP1A1 wt/wt and wt/vt + vt/vt groups comparing those in the highest tertile of years smoked to the lowest tertile, respectively.

Discussion

The exon 7 polymorphism is believed to cause an increase in the aryl hydrocarbon hydroxylase activity of CYP1A1 [2]. Although the CYP1A1 variant has not been linked with elevated PAH-DNA adducts, it has been linked to an increased rate of p53 mutations in human lung carcinomas (8). The hypothesis that increased bioactivation of cigarette smoke carcinogens by CYP1A1 leads to higher susceptibility to lung cancer is biologically plausible.

There is evidence of higher prevalence of the CYP1A1 val/val homozygote in Japanese lung cancer cases compared to controls (9). In non-Asian populations the association between the polymorphisms of CYP1A1 and lung cancer risk are not consistent. It is likely that the currently recognized CYP1A1 and other genetic polymorphisms are only a fraction of those that influence the bioactivation of carcinogens. It is also worth noting that due to the cross sectional nature of most prior reports, only the prevalence of a particular genotype in their respective populations with lung cancer could be ascertained, and not lung cancer risk per se.

In the current study, we investigated the association between the exon 7 adenine to guanine substitution at residue 462 of CYP1A1 and lung cancer risk. There was no overall association between the combined homozygous isoleucine to valine variant and heterozygous CYP1A1 genotype and lung cancer risk. Further , the CYP1A1 genotype did not appear to modify lung cancer risk associated with cumulative cigarette smoking overall. CYP1A1 genotype did not also greatly modify the risk of lung cancer due to smoking duration stratified by beta-carotene supplementation.

However, among individuals not supplemented with alpha-tocopherol, the CYP1A1 variant allele appeared to modify the lung cancer-smoking association with dramatically increased risk (>20- fold) for the CYP1A1 variant allele in the highest tertile of years smoked, compared to the reference group. By contrast, CYP1A1 genotype did not modify lung cancer risk associated with cigarette smoking among those supplemented with alpha-tocopherol. These findings suggest that alpha-tocopherol supplementation reduces the risk of lung cancer associated with cumulative smoking exposure regardless of CYP1A1 genotype. However, the reduction in lung cancer risk was greatest among individuals with the CYP1A1 variant allele. Our findings also suggested that alpha-tocopherol is able to ameliorate the smoking associated risk of lung cancer. This finding is consistent with the ATBC study finding showing a lower rate of lung cancer associated with years of smoking among individuals receiving supplemental alpha-tocopherol (4).

The mechanisms by which alpha-tocopherol reduces lung cancer risk are generally speculative. It is possible that alpha-tocopherol inhibits the mechanisms of lung cancer development by acting as a scavenger of oxidative free radicals [10]. This may be particularly important among individuals with the CYP1A1 variant allele. In addition, alpha-tocopherol may increase cellular glutathione levels and consequently increasing the rate of carcinogen detoxification (11). Furthermore, alpha-tocopherol may also stimulate the phase II detoxification enzymes such as the glutathione-s- transferases (GSTs). For example, alpha-tocopherol supplementation has been shown to induce the expression of GSTs in rats (12).

In addition, in another study nested within the ATBC cohort, the GST-M1 hull genotype (phase II enzyme glutathione s-tranferase absent) appeared to increase the lung cancer risk associated with cumulative tobacco exposure whilst alpha-tocopherol apparently reduced this risk (13). This observation and the observations of the current study suggest that the mechanism of action of alpha-tocopherol is probably pathway specific (Phase I and II enzyme pathway specific) and not enzyme specific.

One explanation for the finding of no association between the CYP1A1 variant allele and lung cancer may be that our study population consisted of heavy smokers, among whom any modulating influence of the genotype may have been overwhelmed by the carcinogen exposure. Alternatively, other genotypes, such as the phase II detoxification enzyme GST, may need to be taken into account. In several studies lung cancer risk was higher when both the CYP1A1 and GSTM1 genotype were considered than for either genotype alone (14).

Generally, studies evaluating the association between the CYP1A1 exon 7 polymorphism and lung cancer have had small sample size with cases ascertained retrospectively, and due to their cross sectional nature the risk conferred by genotype could not be assessed. However, in our study the large sample size and prospective blood collection provided us with sufficient sample size and the ability to assess risk due

to genotype. The main limitation of our study was the almost complete unavailability of the "informative" homozygous variant CYP1A1 genotype and the low prevalence of the variant allele that greatly reduced power in the stratified analyses. Another limitation was the unavailability of an ideal reference group of non-smokers or light smokers. This may have contributed to our finding of no association between CYP1A1 genotype and risk of lung cancer.

In summary, we show that the CYP1A1 variant allele does not increase the risk of lung cancer among male smokers from Finland, however, alpha-tocopherol may reduce the risk of lung cancer associated with cumulative tobacco exposure and may be even more important when the individual has an inherited CYP1A1 isoleucine to valine polymorphism.

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